Remarks

Claims 45, 46, 59-60 and 63-65 are currently amended. Support for amendment to claims 45 and 63 can be found in Table 1 of the instant specification. Claims 59 and 60 are amended to recite correct antecedent basis. Claims 46 and 64 are amended to correct typographical errors.

Claims 66-101 are added. Support for new claims 66-85 can be found in Table 1 of the instant specification. Support for new claims 86-101 can be found on page 14, lines 17-29.

The Examiner is authorized to charge Deposit Account No. 23/2825 for any additional claims fees due.

No new matter has been added.

Claims 1, 10, 11, 15, 20-24, 34, 41-55 and 59-101 are pending.

Claim Rejections Under 35 U.S.C. § 112, second paragraph

Claims 45 and 63 are rejected under 35 U.S.C. § 112, second paragraph, as being vague and indefinite in the recitation of "chemokines of Table 1". Applicants have amended claims 45 and 63 to recite the chemokines listed in Table 1.

In view of this amendment, Applicants respectfully request that the Examiner reconsider and withdraw the rejection under 35 U.S.C. § 112, second paragraph.

Claim Rejections Under 35 U.S.C. § 112, first paragraph

Claims 10, 11, 59 and 60 are rejected under 35 U.S.C. § 112, first paragraph, for failing to comply with the written description requirement. According to the Examiner, chemokines having agonist and antagonist activities are not sufficient described. In particular, the Examiner states that the specification does not provide a structure of either a chemokine altered at the amino terminus to produce a chemokine antagonist or of a truncated chemokine that is a chemokine agonist. Applicants respectfully traverse.

Applicants wish to point out that the terms "agonist" and "antagonist" refer to functional art-recognized categories of chemokines. The specification teaches that naturally occurring chemokines may exhibit agonist or antagonist activity. (See for example page 17, lines 8-16, where it is taught that chemokine agonist activity is the ability of a chemokine to bind to its cognate receptor and activate the receptor and that chemokine antagonist activity is the ability of

a chemokine to bind to its cognate receptor without activating the receptor and triggering intracellular signaling events.) Agonists and antagonists thus include naturally occurring chemokines which may be full-length, such as those listed in Table 1, as evidenced by the some of the literature abstracts attached hereto as Appendix A.

Chemokine agonists and antagonists also include modified versions of full length chemokines (e.g., truncated or elongated chemokines). The specification provides methods for generating agonists and antagonists (e.g., by modification of amino or carboxy termini) and cites at least two references that disclose such compounds (i.e., Hechtman et al. J. Immunol. 147(3):883-892 (1991) and Gog et al. J. Exp. Med. 186:131-137 (1997)). At the time of filing, the art was familiar with the generation of chemokine agonists and antagonists (e.g., Gong et al., J. Exp. Med., 181: 631-640 (1995) show that truncations of the amino terminus of monocyte chemoattractant protein (MCP-1) result in chemokines with antagonist properties; Proudfoot et al., J. Biol. Chem., 274: 32478-32485 (1999) report that amino-terminally modified RANTES analogues demonstrate differential effects on RANTES receptors; and Proost et al., J. of Immunol., 160: 4034-4041 (1998) show the effects of posttranslational modifications on the activity of MCP-1 and MCP-2 and identify amino-terminally truncated MCP-2 as a natural chemokine inhibitor). Further evidence of the art's familiarity with modified chemokines as agonists and antagonists can be found in the literature abstracts submitted in Appendix A.

Unlike the case law cited by the Examiner, various chemokine agonists and antagonists had already been chemically or structurally identified by the art at the time of filing. These include naturally occurring full length chemokines, naturally occurring modified chemokines, and synthetic modified chemokines. In other words, there is structural basis and structural similarity within the chemokine agonist and antagonist genera, as presently claimed.

Applicants reiterate that the claimed invention relates to a composition of a biotinylated chemokine that is pharmacologically active, optionally complexed with an anti-biotin antibody. The Examiner rightly does not challenge the genus of "chemokines", particularly since the meaning of that genus is clear to those of ordinary skill in the art based on the knowledge in the art at the time of filing and the teaching in the specification. In a similar manner, the genera of chemokine agonists and chemokine antagonists are also clear to those of ordinary skill in the art based on the knowledge in the art at the time of filing and the teaching in the specification.

In view of the foregoing, Applicants respectfully request that the Examiner reconsider and withdraw the rejection under 35 U.S.C. § 112, first paragraph.

Claim Rejections Under 35 U.S.C. § 112, first paragraph

The Examiner has rejected claims 1, 10, 11, 15, 20-24, 34, 41-55, and 59-65 under 35 U.S.C. § 112, first paragraph, for failing to comply with the enablement requirement. According to the Examiner, "the specification does not teach how to prevent the elimination of the instant antibody-antigen complexes from circulation in order to attain an increased half-life over the biotinylated chemokine as disclosed". The Examiner concludes that "one of skill in the art would be subject to undue experimentation in order to make complexes having an increased half-life and use the claimed complex to produce a therapeutic effect". The Examiner bases the rejection on references that show that antibody-antigen complexes are rapidly eliminated from the circulation. Applicants respectfully traverse for the reasons stated below.

First, the claimed invention relates to compositions of biotinylated pharmacologically active chemokines, optionally complexed with anti-biotin antibodies. While the Examiner's bases her rejection on chemokine half-life limitations, these limitations are found only in claims 15, 48 and 49. Accordingly, the rejection of claims 10, 11, 20-24, 34, 41-47, 50-55 and 59-65 at a minimum should be withdrawn.

Second, as stated in an earlier response, the enablement requirement for a composition claim is satisfied by a specification that teaches how to make and use the composition. The pending composition claims are not limited in their use; thus, any "real-world" use is sufficient for enablement, regardless of whether that use requires an increased half-life or not. Applicants have provided various uses of the claimed compositions as discussed in an earlier response (to which the Examiner is directed), and these uses are not solely limited to therapeutic use, as incorrectly indicated by the Examiner in paragraph 5 of the pending action.

Finally, and notwithstanding the above, the invention is premised *in part* on the unexpected discovery that biotinylated chemokines are cleared from the circulation more slowly when coupled to anti-biotin antibodies, as compared to free uncomplexed chemokines. This is evidenced by the specification and the working examples provided therein. See for example page 8, lines 7-10, and page 18, lines 8-11. Significantly, the Examples demonstrate that biotinylated chemokines complexed with anti-biotin antibodies functioned in vivo for periods of

time far in excess of biotinylated chemokines alone (as evidenced by inhibition of neutrophil and lymphocyte recruitment at 48 and 72 hours post challenge). The Examiner appears to have either overlooked the working examples or to have given them little weight. Instead, the Examiner focused solely on another Wands factor (i.e., the state of the art). The Examiner should consider the Wands factors in their totality, including actual working examples, in determining whether undue experimentation is required to make and use the claimed invention. Furthermore, if the Examiner did consider the working examples, then she has the burden of explaining why those examples and the corresponding data are doubted as evidence of enablement of increased half-life of antibody-complexed biotinylated chemokines.

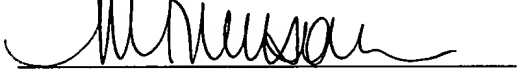
Applicants respectfully request that the Examiner reconsider and withdraw the rejection under 35 U.S.C. § 112, first paragraph.

Summary

Applicants believe that each of the pending claims now is in condition for allowance. Applicants respectfully request that the Examiner telephone the undersigned in the event that the claims are not found to be in condition for allowance.

If the Examiner has any questions and believes that a telephone conference with the undersigned would prove helpful in expediting the prosecution of this application, the Examiner is urged to call the undersigned at (617) 646-8000 (extension 8266).

Respectfully submitted,
Walter Newman et al., Applicants



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Docket No.: L0559.70001US00
Date: August 19, 2004
x08.19.04x

Appendix A — 1

1: Blood. 2000 Feb 15;95(4):1151-7.

KSHV-encoded CC chemokine vMIP-III is a CCR4 agonist, stimulates angiogenesis, and selectively chemoattracts TH2 cells.

Stine JT, Wood C, Hill M, Epp A, Raport CJ, Schweickart VL, Endo Y, Sasaki T, Simmons G, Boshoff C, Clapham P, Chang Y, Moore P, Gray PW, Chantry D.

ICOS Corporation, Bothell, WA 98021, USA.

Kaposi's sarcoma-associated herpesvirus (KSHV) encodes 3 genes that are homologous to cellular chemokines. vMIP-III, the product of open reading frame K4.1, is the most distantly related to human chemokines and has yet to be characterized. We have examined the interaction of vMIP-III with chemokine receptors, its expression in KS lesions, and its in ovo angiogenic properties. We show expression of vMIP-III in KS lesions and demonstrate the stimulation of angiogenesis by this chemokine, like vMIP-I and vMIP-II, in the chick chorioallantoic membrane assay. vMIP-III does not block human immunodeficiency virus entry through the coreceptors CCR3, CCR5, or CXCR4. However, vMIP-III is an agonist for the cellular chemokine receptor CCR4. CCR4 is expressed by TH2-type T cells. Consistent with this, vMIP-III preferentially chemoattracts this cell type. Because of these biologic properties and because it is expressed in KS lesions, vMIP-III may play an important role in the pathobiology of KS. (Blood. 2000;95:1151-1157)

PMID: 10666184 [PubMed - indexed for MEDLINE]

2

1: J Biol Chem. 1999 Jun 18;274(25):17478-83.

LD78beta, a non-allelic variant of human MIP-1alpha (LD78alpha), has enhanced receptor interactions and potent HIV suppressive activity.

Nibbs RJ, Yang J, Landau NR, Mao JH, Graham GJ.

Beatson Institute for Cancer Research, CRC Beatson Laboratories, Garscube Estate, Switchback Road, Bearsden, Glasgow, G61 1BD Scotland, United Kingdom.

Chemokines play diverse roles in inflammatory and non-inflammatory situations via activation of heptahelical G-protein-coupled receptors. Also, many chemokine receptors can act as cofactors for cellular entry of human immunodeficiency virus (HIV) in vitro. CCR5, a receptor for chemokines MIP-1alpha (LD78alpha), MIP-1beta, RANTES, and MCP2, is of particular importance in vivo as polymorphisms in this gene affect HIV infection and rate of progression to AIDS. Moreover, the CCR5 ligands can prevent HIV entry through this receptor and likely contribute to the control of HIV infection. Here we show that a non-allelic isoform of human MIP-1alpha (LD78alpha), termed LD78beta or MIP-1alphaP, has enhanced receptor binding affinities to CCR5 (approximately 6-fold) and the promiscuous beta-chemokine receptor, D6 (approximately 15-20-fold). We demonstrate that a proline residue at position 2 of MIP-1alphaP is responsible for this enhanced activity. Moreover, MIP-1alphaP is by far the most potent natural CCR5 agonist described to date, and importantly, displays markedly higher HIV1 suppressive activity than all other human MIP-1alpha isoforms examined. In addition, while RANTES has been described as the most potent inhibitor of CCR5-mediated HIV entry, MIP-1alphaP was as potent as, if not more potent than, RANTES in HIV-1 suppressive assays. This property suggests that MIP-1alphaP may be of importance in controlling viral spread in HIV-infected individuals.

PMID: 10364178 [PubMed - indexed for MEDLINE]

3

1: J Clin Invest. 1999 Aug;104(4):R1-5.

The LD78beta isoform of MIP-1alpha is the most potent CCR5 agonist, and HIV-1-inhibiting chemokine.

Menten P, Struyf S, Schutyser E, Wuyts A, De Clercq E, Schols D, Proost P, Van Damme J.

Laboratory of Molecular Immunology, Rega Institute for Medical Research, University of Leuven, B-3000 Leuven, Belgium.

LD78alpha and LD78beta are 2 highly related nonallelic genes that code for different isoforms of the human CC chemokine macrophage inflammatory protein-1alpha (MIP-1alpha). Two molecular forms of natural LD78beta (7.778 and 7.793 kDa) were identified from conditioned media of stimulated peripheral blood mononuclear cells. Although LD78alpha and LD78beta only differ in 3 amino acids, both LD78beta variants were 100-fold more potent chemoattractants for mouse lymphocytes than was LD78alpha. On the contrary, LD78beta was only 2-fold more efficient than LD78alpha in chemoattracting human lymphocytes and monocytes. Using CC chemokine receptor-transfected cells, both molecular forms of LD78beta proved to be much more potent than LD78alpha in inducing an intracellular calcium rise through CCR5. Compared with LD78alpha and RANTES, this preferential binding of LD78beta to CCR5 resulted in a 10- to 50-fold higher potency in inhibiting infection of peripheral blood mononuclear cells by CCR5-using (R5) HIV-1 strains. To date, LD78beta is the most potent chemokine for inhibiting HIV-1 infection, and can be considered as a potentially important drug candidate for the treatment of infection with R5 HIV-1 strains.

PMID: 10449444 [PubMed - indexed for MEDLINE]

4

1: J Biol Chem. 1999 Jul 30;274(31):21569-74.

HHV8-encoded vMIP-I selectively engages chemokine receptor CCR8. Agonist and antagonist profiles of viral chemokines.

Dairaghi DJ, Fan RA, McMaster BE, Hanley MR, Schall TJ.

Divisions of Discovery Biology and Molecular Pharmacology, ChemoCentryx, San Carlos, California 94070, USA.

Uncertainty regarding viral chemokine function is mirrored by an incomplete knowledge of host chemokine receptor usage by the virally encoded proteins. One such molecule is vMIP-I, a C-C type chemokine of undefined function and binding specificity, encoded by the Kaposi's sarcoma herpesvirus HHV-8. We report here that vMIP-I binds to and induces cytosolic [Ca(2+)] signals in human T cells selectively through CCR8, a CC chemokine receptor associated with Th2 lymphocytes. Furthermore, using a panel of 65 different human, viral, and rodent chemokines, we have established a comprehensive ligand binding "fingerprint" for CCR8. The receptor exhibits marked "high" affinity (K(d) < 15 nM) only for four chemokines, three of them of viral origin: vMIP-I, vMIP-II, vMCC-I, and human I-309. A previously unreported second class of lower affinity ligands includes MCP-3 and possibly two other viral chemokines. vMIP-I and I-309 appear to act as CCR8 agonists: binding to and inducing cytosolic [Ca(2+)] elevation through the receptor. By contrast, vMIP-II and vMCC-I act as potent antagonists: binding without inducing signaling, and blocking the effects of I-309 and vMIP-I. These results suggest a ligand hierarchy for CCR8, identifying vMIP-I as a selective viral chemokine agonist. CCR8 may thus engage a specific subset of chemokines with the potential to regulate each other during viral infection and immune regulation.

PMID: 10419462 [PubMed - indexed for MEDLINE]

5

1: J Exp Med. 1999 Jun 21;189(12):1993-8.

The Kaposi's sarcoma-related herpesvirus (KSHV)-encoded chemokine vMIP-I is a specific agonist for the CC chemokine receptor (CCR)8.

Endres MJ, Garlisi CG, Xiao H, Shan L, Hedrick JA.

Department of Antiviral Therapy, Schering-Plough Research Institute, Kenilworth, New Jersey 07033, USA.

The Kaposi's sarcoma-related herpesvirus (KSHV), also designated human herpesvirus 8, is the presumed etiologic agent of Kaposi's sarcoma and certain lymphomas. Although KSHV encodes several chemokine homologues (viral macrophage inflammatory protein [vMIP]-I, -II, and -III), only vMIP-II has been functionally characterized. We report here that vMIP-I is a specific agonist for the CC chemokine receptor (CCR)8 that is preferentially expressed on Th2 T cells. Y3 cells transfected with CCR8 produced a calcium flux in response to vMIP-I and responded vigorously in in vitro chemotaxis assays. In competition binding experiments, the interaction of vMIP-I with CCR8 was shown to be specific and of high affinity. In contrast to its agonist activity at CCR8, vMIP-I did not interact with CCR5 or any of 11 other receptors examined. Furthermore, vMIP-I was unable to inhibit CCR5-mediated HIV infection. These findings suggest that expression of vMIP-I by KSHV may influence the Th1/Th2 balance of the host immune response.

PMID: 10377196 [PubMed - indexed for MEDLINE]

6

1: Blood. 1999 Sep 15;94(6):1899-905.

CCR5 binds multiple CC-chemokines: MCP-3 acts as a natural antagonist.

Blanpain C, Migeotte I, Lee B, Vakili J, Doranz BJ, Govaerts C, Vassart G, Doms RW, Parmentier M.

IRIBHN and Service de Genetique Medicale, Universite Libre de Bruxelles, Campus Erasme, Bruxelles, Belgium.

CCR5 was first characterized as a receptor for MIP-1alpha, MIP-1beta, and RANTES, and was rapidly shown to be the main coreceptor for M-tropic human immunodeficiency virus (HIV)-1 strains and simian immunodeficiency virus (SIV). Chemokines constitute a rapidly growing family of proteins and receptor-chemokine interactions are known to be promiscuous and redundant. We have therefore tested whether other CC-chemokines could bind to and activate CCR5. All CC-chemokines currently available were tested for their ability to compete with [(125)I]-MIP-1beta binding on a stable cell line expressing recombinant CCR5, and/or to induce a functional response in these cells. We found that in addition to MIP-1beta, MIP-1alpha, and RANTES, five other CC-chemokines could compete for [(125)I]-MIP-1beta binding: MCP-2, MCP-3, MCP-4, MCP-1, and eotaxin binding was characterized by IC(50) values of 0.22, 2.14, 5.89, 29.9, and 21.7 nmol/L, respectively. Among these ligands, MCP-3 had the remarkable property of binding CCR5 with high affinity without eliciting a functional response, MCP-3 could also inhibit the activation of CCR5 by MIP-1beta and may therefore be considered as a natural antagonist for CCR5. It was unable to induce significant endocytosis of the receptor. Chemokines that could compete with high affinity for MIP-1beta binding could also compete for monomeric gp120 binding, although with variable potencies; maximal gp120 binding inhibition was 80% for MCP-2, but only 30% for MIP-1beta. MCP-3 could compete efficiently for gp120 binding but was, however, found to be a weak inhibitor of HIV infection, probably as a consequence of its inability to downregulate the receptor.

PMID: 10477718 [PubMed - indexed for MEDLINE]

7

1: J Biol Chem. 1999 Nov 5;274(45):32478-85.

Amino-terminally modified RANTES analogues demonstrate differential effects on RANTES receptors.

Proudfoot AE, Buser R, Borlat F, Alouani S, Soler D, Offord RE, Schroder JM, Power CA, Wells TN.

Serono Pharmaceutical Research Institute, 14 Chemin des Aulx, 1228 Plan-les-Ouates, Geneva, Switzerland. amanda.proudfoot@serono.com

Modification of the amino terminus of regulated on activated normal T-cell expressed (RANTES) has been shown to have a significant effect on biological activity and produces proteins with antagonist properties. Two amino-terminally modified RANTES proteins, Met-RANTES and aminooxypentane-RANTES (AOP-RANTES), exhibit differential inhibitory properties on both monocyte and eosinophil chemotaxis. We have investigated their binding properties as well as their ability to activate the RANTES receptors CCR1, CCR3, and CCR5 in cell lines overexpressing these receptors. We show that Met-RANTES has weak activity in eliciting a calcium response in Chinese hamster ovary cells expressing CCR1, CCR3, and CCR5, whereas AOP-RANTES has full agonist activity on CCR5 but is less effective on CCR3 and CCR1. Their ability to induce chemotaxis of the murine pre-B lymphoma cell line, L1.2, transfected with the same receptors, consolidates these results. Monocytes have detectable mRNA for CCR1, CCR2, CCR3, CCR4, and CCR5, and they respond to the ligands for these receptors in chemotaxis but not always in calcium mobilization. AOP-RANTES does not induce calcium mobilization in circulating monocytes but is able to do so as these cells acquire the macrophage phenotype, which coincides with a concomitant up-regulation of CCR5. We have also tested the ability of both modified proteins to induce chemotaxis of freshly isolated monocytes and eosinophils. Cells from most donors do not respond, but occasionally cells from a particular donor do respond, particularly to AOP-RANTES. We therefore hypothesize that the occasional activity of AOP-RANTES to induce leukocyte chemotaxis is due to donor to donor variation of receptor expression.

PMID: 10542293 [PubMed - indexed for MEDLINE]

8

1: Kidney Int. 1999 Dec;56(6):2107-15.

The chemokine receptor antagonist AOP-RANTES reduces monocyte infiltration in experimental glomerulonephritis.

Panzer U, Schneider A, Wilken J, Thompson DA, Kent SB, Stahl RA.

Department of Medicine, Division of Hamburg, University of Hamburg, Germany.

The chemokine receptor antagonist AOP-RANTES reduces monocyte infiltration in experimental glomerulonephritis. BACKGROUND: This study was designed to evaluate the role of the novel chemokine receptor antagonist amino-oxypentane RANTES (AOP-RANTES), which blocks the binding of macrophage inflammatory protein-1alpha (MIP-1alpha), MIP-1beta, and RANTES to the chemokine receptor-5 (CCR-5) on the infiltration of monocytes in experimental glomerulonephritis. METHODS: Rats were treated twice daily with 12.5 microg AOP-RANTES following an induction of anti-rat-thymocyte antibody-mediated glomerulonephritis. The white blood cell count, glomerular monocyte infiltration, chemokine expression, and collagen type IV deposition were assessed. RESULTS: The induction of glomerulonephritis increased glomerular monocyte/macrophage (M/M) infiltration at 24 hours and at 5 days was still higher than in controls. AOP-RANTES prevented glomerular M/M infiltration at 24 hours and at 5 days. This was paralleled by reduced glomerular collagen type IV deposition as a fibrotic marker in nephritic animals. CONCLUSION: These data show that the CCR-5 chemokine receptor antagonist AOP-RANTES ameliorates M/M infiltration and improves glomerular pathology in experimental glomerulonephritis. The use of chemokine receptor antagonists may offer a new therapeutic option in inflammatory renal injuries.

PMID: 10594786 [PubMed - indexed for MEDLINE]

9

1: J Immunol. 2000 Feb 1;164(3):1488-97.

C-C chemokine receptor 3 antagonism by the beta-chemokine macrophage inflammatory protein 4, a property strongly enhanced by an amino-terminal alanine-methionine swap.

Nibbs RJ, Salcedo TW, Campbell JD, Yao XT, Li Y, Nardelli B, Olsen HS, Morris TS, Proudfoot AE, Patel VP, Graham GJ.

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Allergic reactions are characterized by the infiltration of tissues by activated eosinophils, Th2 lymphocytes, and basophils. The beta-chemokine receptor CCR3, which recognizes the ligands eotaxin, eotaxin-2, monocyte chemotactic protein (MCP) 3, MCP4, and RANTES, plays a central role in this process, and antagonists to this receptor could have potential therapeutic use in the treatment of allergy. We describe here a potent and specific CCR3 antagonist, called Met-chemokine beta 7 (Ckbeta7), that prevents signaling through this receptor and, at concentrations as low as 1 nM, can block eosinophil chemotaxis induced by the most potent CCR3 ligands. Met-Ckbeta7 is a more potent CCR3 antagonist than Met- and aminooxypentane (AOP)-RANTES and, unlike these proteins, exhibits no partial agonist activity and is highly specific for CCR3. Thus, this antagonist may be of use in ameliorating leukocyte infiltration associated with allergic inflammation. Met-Ckbeta7 is a modified form of the beta-chemokine macrophage inflammatory protein (MIP) 4 (alternatively called pulmonary and activation-regulated chemokine (PARC), alternative macrophage activation-associated C-C chemokine (AMAC) 1, or dendritic cell-derived C-C chemokine (DCCK) 1). Surprisingly, the unmodified MIP4 protein, which is known to act as a T cell chemoattractant, also exhibits this CCR3 antagonistic activity, although to a lesser extent than Met-Ckbeta7, but to a level that may be of physiological relevance. MIP4 may therefore use chemokine receptor agonism and antagonism to control leukocyte movement in vivo. The enhanced activity of Met-Ckbeta7 is due to the alteration of the extreme N-terminal residue from an alanine to a methionine.

PMID: 10640766 [PubMed - indexed for MEDLINE]

10

1: J Immunol. 1998 Apr 15;160(8):4034-41.

Posttranslational modifications affect the activity of the human monocyte chemotactic proteins MCP-1 and MCP-2: identification of MCP-2(6-76) as a natural chemokine inhibitor.

Proost P, Struyf S, Couvreur M, Lenaerts JP, Conings R, Menten P, Verhaert P, Wuyts A, Van Damme J.

Rega Institute for Medical Research, Laboratory of Molecular Immunology, University of Leuven, Belgium.

Chemokines are important mediators in infection and inflammation. The monocyte chemotactic proteins (MCPs) form a subclass of structurally related C-C chemokines. MCPs select specific target cells due to binding to a distinct set of chemokine receptors. Recombinant and synthetic MCP-1 variants have been shown to function as chemokine antagonists. In this study, posttranslationally modified immunoreactive MCP-1 and MCP-2 were isolated from mononuclear cells. Natural forms of MCP-1 and MCP-2 were biochemically identified by Edman degradation and mass spectrometry and functionally characterized in chemotaxis and Ca2+-mobilization assays. Glycosylated MCP-1 (12 and 13.5 kDa) was found to be two- to threefold less chemotactic for monocytes and THP-1 cells than nonglycosylated MCP-1 (10 kDa). Natural, NH2-terminally truncated MCP-1(5-76) and MCP-1(6-76) were practically devoid of bioactivity, whereas COOH-terminally processed MCP-1(1-69) fully retained its chemotactic and Ca2+-inducing capacity. The capability of naturally modified MCP-1 forms to desensitize the Ca2+ response induced by intact MCP-1 in THP-1 cells correlated with their agonistic potency. In contrast, naturally modified MCP-2(6-76) was devoid of activity, but could completely block the chemotactic effect of intact MCP-2 as well as that of MCP-1, MCP-3, and RANTES. Carboxyl-terminally processed MCP-2(1-74) did retain its chemotactic potency. Although comparable as a chemoattractant, natural intact MCP-2 was found to be 10-fold less potent than MCP-1 in inducing an intracellular Ca2+ increase. It can be concluded that under physiologic or pathologic conditions, posttranslational modification affects chemokine potency and that natural MCP-2(6-76) is a functional C-C chemokine inhibitor that might be useful as an inhibitor of inflammation.

PMID: 9558113 [PubMed - indexed for MEDLINE]

11

1: J Biol Chem. 1999 Nov 5;274(45):32478-85.

Amino-terminally modified RANTES analogues demonstrate differential effects on RANTES receptors.

Proudfoot AE, Buser R, Borlat F, Alouani S, Soler D, Offord RE, Schroder JM, Power CA, Wells TN.

Serono Pharmaceutical Research Institute, 14 Chemin des Aulx, 1228 Plan-les-Ouates, Geneva, Switzerland. amanda.proudfoot@serono.com

Modification of the amino terminus of regulated on activated normal T-cell expressed (RANTES) has been shown to have a significant effect on biological activity and produces proteins with antagonist properties. Two amino-terminally modified RANTES proteins, Met-RANTES and aminooxypentane-RANTES (AOP-RANTES), exhibit differential inhibitory properties on both monocyte and eosinophil chemotaxis. We have investigated their binding properties as well as their ability to activate the RANTES receptors CCR1, CCR3, and CCR5 in cell lines overexpressing these receptors. We show that Met-RANTES has weak activity in eliciting a calcium response in Chinese hamster ovary cells expressing CCR1, CCR3, and CCR5, whereas AOP-RANTES has full agonist activity on CCR5 but is less effective on CCR3 and CCR1. Their ability to induce chemotaxis of the murine pre-B lymphoma cell line, L1.2, transfected with the same receptors, consolidates these results. Monocytes have detectable mRNA for CCR1, CCR2, CCR3, CCR4, and CCR5, and they respond to the ligands for these receptors in chemotaxis but not always in calcium mobilization. AOP-RANTES does not induce calcium mobilization in circulating monocytes but is able to do so as these cells acquire the macrophage phenotype, which coincides with a concomitant up-regulation of CCR5. We have also tested the ability of both modified proteins to induce chemotaxis of freshly isolated monocytes and eosinophils. Cells from most donors do not respond, but occasionally cells from a particular donor do respond, particularly to AOP-RANTES. We therefore hypothesize that the occasional activity of AOP-RANTES to induce leukocyte chemotaxis is due to donor to donor variation of receptor expression.

PMID: 10542293 [PubMed - indexed for MEDLINE]

12

1: J Exp Med. 1995 Feb 1;181(2):631-40.

Antagonists of monocyte chemoattractant protein 1 identified by modification of functionally critical NH2-terminal residues.

Gong JH, Clark-Lewis I.

Biomedical Research Centre, University of British Columbia, Vancouver, Canada.

Monocyte chemoattractant protein (MCP)-1 analogues were designed to determine the role of the NH2-terminal region in structure and function. The NH2-terminal residue was important for function and receptor binding, as it could not be deleted or extended. However the NH2-terminal pyroglutamate residue of the wild type was not essential as it could be replaced by several other noncyclic amino acids without loss of activity. Residues 7-10 were essential for receptor desensitization, but were not sufficient for function, and the integrity of residues 1-6 were required for functional activity. A peptide corresponding to MCP-1, 1-10 lacked detectable receptor-binding activities, indicating that residues 1-10 are essential for MCP-1 function, but that other residues are also involved. Several truncated analogues, including 8-76, 9-76, and 10-76, desensitized MCP-1-induced Ca2+ induction, but were not significantly active. These analogues were antagonists of MCP-1 activity with the most potent being the 9-76 analogue (IC50 = 20 nM) The 9-76 specifically bound to MCP-1 receptors with a Kd of 8.3 nM, which was three-fold higher than MCP-1 (Kd 2.8 nM). The 9-76 analogue desensitized the Ca2+ response to MCP-1 and MCP-3, but not to other CC chemokines, suggesting that it is MCP receptor specific. The availability of these compounds will be helpful in evaluating MCP receptor antagonists as anti-inflammatory therapeutics.

PMID: 7836918 [PubMed - indexed for MEDLINE]

B

1: J Leukoc Biol. 1995 May;57(5):703-11.

Structure-activity relationships of chemokines.

Clark-Lewis I, Kim KS, Rajarathnam K, Gong JH, Dewald B, Moser B, Baggiolini M, Sykes BD.

Biomedical Research Centre, University of British Columbia, Vancouver, Canada.

Structural analysis of chemokines has revealed that the alpha/beta structural-fold is highly conserved among both the CXC and CC chemokine classes. Although dimerization and aggregation is often observed, the chemokines function as monomers. The critical receptor binding regions are in the NH2-terminal 20 residues of the protein and are the least ordered in solution. The flexible NH2-terminal region is the most critical receptor binding site and a second site also exists in the loop that follows the two disulfides. The well-ordered regions are not directly involved in receptor binding but, along with the disulfides, they provide a scaffold that determines the conformation of the sites that are critical for receptor binding. These general requirements for function are common to all the chemokines. For the CC chemokines, receptor activation and receptor binding regions are separate within the 10 residue NH2-terminal region. This has allowed identification of high affinity analogs that do not activate the receptor and are potent antagonists.

Publication Types:
Review
Review, Academic

PMID: 7759949 [PubMed - indexed for MEDLINE]

14

1: J Exp Med. 1996 Feb 1;183(2):681-5.

Deletion of the NH2-terminal residue converts monocyte chemotactic protein 1 from an activator of basophil mediator release to an eosinophil chemoattractant.

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Chemotactic cytokines of the CC subfamily (CC chemokines) are considered as major mediators of allergic inflammation owing their actions on basophil and eosinophil leukocytes. The monocyte chemotactic protein (MCP) 1 is a potent inducer of mediator release from basophils but is inactive on eosinophils. To obtain information on the structural determinants of the activities of MCP-1, we have synthesized several NH2-terminally truncated analogues and tested their effects on basophils and eosinophils. Through deletion of the NH2-terminal residue, MCP-1(2-76) was obtained, which was a potent activator of eosinophils, as assessed by chemotaxis, cytosolic free Ca2+ changes, actin polymerization, and that induction of the respiratory burst. In contrast, the activity of MCP-1(2-76) on basophil leukocytes was dramatically decreased (50-fold) compared with that of full-length MCP-1. Deletion of the next residue led to total loss of activity on eosinophil and basophil leukocytes. Analogues with three or four residue deletions, MCP-1(4-76) and MCP-1(5-76), were again active on both cells, whereas all further truncation analogues, MCP-1(6-76) through MCP-1(10-76), were inactive. Thus, a minimal structural modification can change receptor and target cell selectivity of MCP-1. Our observations indicate that the recognition sites of CC chemokine receptors on eosinophils and basophils are similar, although they discriminate between MCP-1 and MCP-1(2-76) and suggest NH2-terminal processing as a potential mechanism for the regulation of CC chemokine activities.

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15

1: Methods. 1996 Aug;10(1):126-34.

The Molecular Basis of the Chemokine/Chemokine Receptor Interaction-Scope for Design of Chemokine Antagonists

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Chemokines are a family of small proteins that are present in a variety of inflammatory conditions and have been shown to activate and recruit a wide variety of cell types. They bind to a family of seven transmembrane G-protein-coupled receptors. Models for the interaction of the chemokines with their receptors suggest a two-step mechanism. Initially, the main body of the chemokine interacts with the outside of the receptor (Site 1), and this interaction directs receptor selectivity. Subsequently, the flexible amino-terminus of the chemokine interacts with the receptor core (Site 2) to initiate the signaling response. Mutagenesis studies of IL-8, the archetypal CXC chemokine, show that altering the protein on the third beta-sheet can change the receptor selectivity from that of a CXC chemokine and introduce CC chemokine activity-confirming the role of this region in Site 1. Mutagenesis studies of the amino-terminal region of IL-8 showed that a tripeptide, ELR, was essential for the interaction with Site 2. We have shown, using synthetic peptides and site-directed mutagenesis, that the amino-terminus of RANTES is important in the signaling response (Site 2). Mutations that alter only the interaction with Site 2 are capable of binding the receptor and not signaling and are therefore potential antagonists. Such antagonists have now been made by several groups, for a number of the chemokine receptors, and are active at nanomolar concentrations. These can now be used to test the hypothesis that antagonism of chemokine receptors will lead to a reduction in inflammation in vivo.

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16

1: J Immunol. 1998 Nov 1;161(9):4944-9.

A His19 to Ala mutant of melanoma growth-stimulating activity is a partial antagonist of the CXCR2 receptor.

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Melanoma growth stimulating activity (MGSA) and IL-8 are related chemokines that are potent chemoattractants and activators of neutrophils both in vitro and in vivo. Increasing evidence suggests that these molecules play an important role in inflammation; thus, antagonists of their action could be useful therapeutically as antiinflammatory agents. We have generated an MGSA mutant, H19A, that shows a dissociation between receptor binding and biologic activity. The biologic activity of the H19A mutant is between 133-fold and 282-fold less potent than that of wild-type MGSA measured by three independent assays of neutrophil function, i.e., elastase release chemotaxis and the up-regulation of CD18. In addition, pretreatment of cells with the H19A mutant inhibited the ability of MGSA to induce elastase release and chemotaxis and to increase intracellular calcium. However, competition binding studies in cells transfected with the CXCR2 receptor and in neutrophils demonstrate that the receptor affinity of the H19A mutant is only 13-fold less than that of wild-type MGSA. These studies suggest that the mutant MGSA is defective in activating signaling through the receptor and indicate that binding to the receptor is not sufficient to activate a biologic response. The dissociation between receptor binding and activation for this mutant suggests that it should be possible to design antagonists of MGSA that may be of clinical utility.

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17

1: Int Arch Allergy Immunol. 1999 Feb-Apr;118(2-4):462-5.

The CC chemokine receptor antagonist met-RANTES inhibits eosinophil effector functions.

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Eosinophils play an important role in allergic diseases such as allergic asthma, rhinoconjunctivitis and atopic dermatitis. Recruitement of eosinophils to the side of inflammation, the release of reactive oxygen species, leading to tissue damage, and the propagation of the inflammatory response are mediated by chemokines. Thus, the applicability of agents able to inhibit or antagonize chemokine-induced eosinophil activation seems to be of interest in the treatment of allergic diseases. Therefore, the effect of the CC chemokine antagonist, Met-RANTES, on its effect on human eosinophil effector functions in response to RANTES, MCP-3 and eotaxin was investigated. Met-RANTES had no intrinsic activity on [Ca2+]i transients in eosinophils and was able to dose-dependently inhibit [Ca2+]i transients in eosinophils following stimulation with RANTES, MCP-3 and eotaxin. Besides its effect on [Ca2+]i transients, Met-RANTES dose-dependently inhibited actin polymerization in eosinophils and the release of reactive oxygen species following stimulation with RANTES, MCP-3 and eotaxin. The results of this study lead to the conclusion that Met-RANTES is an effective and powerful compound to antagonize effector functions of human eosinophils following stimulation with RANTES, MCP-3 and eotaxin and is therefore a promising therapeutic approach to prevent the invasion and destructive power of eosinophils in allergic diseases.

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1: J Immunol. 1999 Sep 1;163(5):2829-35.

Inhibition of murine neutrophil recruitment in vivo by CXC chemokine receptor antagonists.

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In this study, we have examined the ability of chemokine receptor antagonists to prevent neutrophil extravasation in the mouse. Two murine CXC chemokines, macrophage-inflammatory protein (MIP)-2 and KC, stimulated the accumulation of leukocytes into s.c. air pouches, although MIP-2 was considerably more potent. The leukocyte infiltrate was almost exclusively neutrophilic in nature. A human CXC chemokine antagonist, growth-related oncogene (GRO)-alpha(8-73), inhibited calcium mobilization induced by MIP-2, but not by platelet-activating factor in leukocytes isolated from the bone marrow, indicating that this antagonist inhibits MIP-2 activity toward murine leukocytes. Pretreatment of mice with GROalpha(8-73) inhibited, in a dose-dependent manner, the MIP-2-induced influx of neutrophils to levels that were not significantly different from control values. Moreover, this antagonist was also effective in inhibiting the leukocyte recruitment induced by TNF-alpha, LPS, and IL-1beta. Leukocyte infiltration into the peritoneal cavity in response to MIP-2 was also inhibited by prior treatment of mice with GROalpha(8-73) or the analogue of platelet factor 4, PF4(9-70). The results of this study indicate 1) that the murine receptor for MIP-2 and KC, muCXCR2, plays a major role in neutrophil recruitment to s.c. tissue and the peritoneal cavity in response to proinflammatory agents and 2) that CXCR2 receptor antagonists prevent acute inflammation in vivo.

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